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Research

A comparative study of simultaneous estimation of cefixime andlevofloxacin by analytical method and their validation

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Abstract

This work is concerned with the simultaneous estimation of Cefixime and Levofloxacin in a marketed drug by UV spectrophotometric method. Methanol as mobile phase. Detection was performed densitometrically at 230 nm and 298 nm. The method was validated for linearity, accuracy, precision, LOD and LOQ and assay.Accuracy (100.6 ± 0. 7 % for Cefixime and 100.7 ± 1.1 % for Levofloxacin) and assay (101.3 % for Cefixime and 101.6% for Levofloxacin)in accordance with ICH guidelines. The method is simple, accurate, and rapid and can therefore be used for routine analysis of both drugs in quality control laboratories.

Keywords: Cefixime, Levofloxacin, Spectrophotometric method, validation.

INTRODUCTION

The United States Pharmacopoeia (USP) states that method validation is done to make sure an analytical approach is precise, specific, repeatable, and robust across the defined range that an analyte will be examined. Method validation is a requirement for regulated laboratories to comply with FDA rules. The FDA identified the requirements in the present version of the USP as those legally recognized when assessing compliance with the Federal Food, Drug, and Cosmetic Act in a 1987 guideline (Guideline for submitting samples and analytical data for methods validation) ([1]). Possibly known as the "eight steps of method validation([11])".

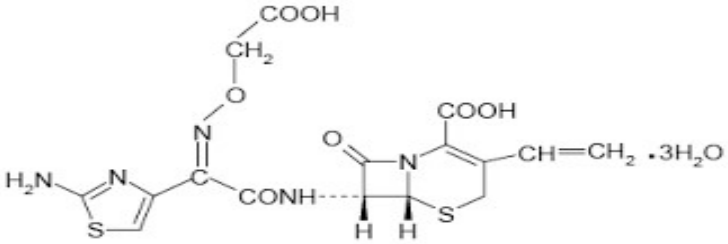
METHOD VALIDATION	Accuracy
	Precision
	Specificity
	Linearity
	Limit of detection

	Limit of quantification
	Robustness
	Ruggedness

Drug profile

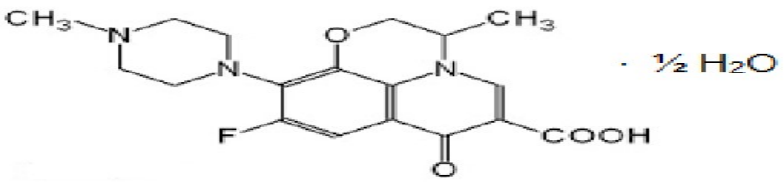
In modern medicine, antibiotics are frequently utilized treatments. Many microorganisms, including bacteria, viruses, fungus, and parasites, have therapeutic significance. The purpose of antibiotics, which are substances that specifically target bacteria, is to treat and prevent bacterial illnesses([4]). The majority of antibiotics used today are created in labs, although they frequently obtain origins from substances discovered in nature. To gain an edge while vying for food, water, or other scarce resources, certain germs, for instance, generate chemicals expressly designed to kill other adjacent bacteria([1]).Cefixime functions by stopping the bacterial protective coating from forming, which is necessary for the survival of the germs in the human body. Levofloxacin stops germs from growing and rebuilding themselves, so that's its mechanism of action works. They successfully treat your infection when combined.Levofloxacin is a broad spectrum antibiotic of the fluoroquinolone drug class([3]). Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections including Gram (-), Gram (+), and atypical bacterial pathogens.Cefiximeis a cephalosporin antibiotic used to treat infections caused by bacteria([2]). These include infections of the: Ear, Nose, sinuses, Throat, Chest and lungs and Urinary system.

Drug profile of Cefixime

Generic name	CefiximeTrihydrate
Structural Formula	
Chemical formula	$C_{16}H_{15}N_5O_7S_2$
Chemical name	(6R,7R)-7-[[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(carboxymethoxyimino)acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid;trihydrate([13]).
Molecular weight	453.45 g / mol
Melting point	218 – 225 ° C
State and solubility	Almost white crystalline powder & slight hygroscopic it is freely soluble in is freely soluble in methanol , chloroform and glacial acetic acid , soluble in ethanol , sparingly soluble in acetic anhydride, slightly soluble in acetone and practically soluble in water([13]).
Category	Third generation orally acting cephalosporin antibiotic.
Mechanism of action	**Inhibitors of cell wall synthesis :-**therefore selectively kill or inhibit bacterial organisms([1]). **Inhibitors of cell membrane function :-** **Inhibitors of protein synthesis :-** Protein synthesis is an essential process necessary for the multiplication and survival of all bacterial cell([5]). **Inhibitors of nucleic acid synthesis :-** inhibit bacterial multiplication and survival. **Inhibitors of other metabolic processes :-** inhibits the DNA & RNA synthesis , thereby stops the multiplication and further growth of bacterial cells([6]).
Contraindication	CefiximeTrihydrate is contraindicated in patients with known hypersensitivity to the drug([3])
Pharmacology	Used to treat bacterial infection. Active against Gram (-) Gram (+) and atypical bacterial pathogens. Used to treat Respiratory tract infections. Used to treat Urinary tract infections. Used to treat abdominal infections.
Indication and usage	CefiximeTrihydrate is indicated in the treatment caused by bacteria. These infections of the ear, nose, sinuses, throat, chest and lungs, also in urinary tract infections.

Adverse reaction	**Nephrotoxicity** : Renal dysfunction. **Dematological** : Rash , Hypersensitivity. **Psychoneurotic** : insomnia, headache, drowsiness, stiffness. **Gastrointestional** : Nausea and vomiting, anorexia, Gastridiscomfort, dry mouth,constipation, diarrhea, abdominal pain, pain stools([2]).

Drug Profile of Levofloxac

Generic name	Levofloxacin Hemihydrate
Chemical formula	$C_{18}H_{20}FN_3O_4$
Structural Formula	
Molecular Weight	361.36 g / mol
Melting Point	224 – 229 ^{0}C
State and Solubility	Yellowish crystalline powder & slight hygroscopie it is freely soluble in methanol, chloroform and glacial acetic acid, soluble in ethanol, sparingly soluble in acetic anhydride. Slightly soluble in acetone and practically in soluble in water([13]).
Category	Anti – bacterial agents, Quinoiones, Nucleic acid aynthesis inhibitors, Anti – infective Agents, Urinary antiseptics.
Mechanism of action	The Flouroquinolone's inhibit the enzyme bacterial DNA gyrase, which nicks double stranded DNA, introduces negative supercoils and then reseals the nicked ends. Therefore stops the further multiplication of the bacterial growth([6]).
Contraindication	Levofloxacin Hemihydrate is contraindicated in patients with known hypersensitivity to the drug.
Pharmacology	Used to treat bacterial infections. Active against Gram (-) Gram (+) and atypical bacterial pathogens. Used to treat Respiratory tract infections. Used to treat Urinary tract infections. Used to treat abdominal infections.
Indication and usage	Levofloxacin Hemihydrate is a broad spectrum antibiotic of the floroquinolone drug class. Its spectrum of activity includes most stains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal and abdominal infections including Gram (-) Gram (+) , and atypical bacterical pathogens([2]).
Indication and usage	Levofloxacin Hemihydrate is a broad spectrum antibiotic of the fluoroquinolone drug class. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal and abdominal infections including Gram (-) Gram (+) , and atypical bacterical pathogens ([2]).

MATERIAL AND METHODS

Apparatus

A double beam UV-visible spectrophotometer (Shimadzu, UV-1700, Japan), attached to a computer software UV probe 2.0, with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells,Analytical balance (CP224S, Sartorius, Germany),
Ultrasonic cleaner (Frontline FS 4, Mumbai, India), Corning volumetric flasks and pipettes of borosilicate glass were used in the study.

Reagents and materials

CefiximeTrihydrate (CEFI) and Levofloxacin Hemihydrate (LEVO) were kindly supplied as a gift samples from Acme Pharmaceuticals, Kherva, Mehsana, Gujarat, India.AR grade methanol (S.D. Fine Chemical Ltd., Mumbai, India.) .Whatman filter paper no. 41 (Whatman International Ltd., England).

Preparation of standard stock solution

Accurately weighed portion of CEFI (10 mg) and LEVO (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentration 100 µg/ml .

Preparation of marketed formulated solution

Twenty tablets were taken, crushed and the powder was weighed. The equivalent weight was taken; the powder with equivalent weight was transferred to 100 ml volumetric flask. And diluted to the mark with methanol .Then pipette out (0.2 ml) and was transferred to 10 ml volumetric flask. Then pipette out 1.0 ml and was transferred to 10 ml volumetric flask. The volume was adjusted with methanol. Final mixture was prepared CEFI (8 µg/ml) and LEVO (10 µg/ml).

METHOD DEVELOPMENT

Determination of wavelength having maximum absorbance

Standard solutions of CEFI (12 µg/ml) and LEVO (12 µg/ml) were scanned in the range of 200 to 400 nm for the determination of wavelength having maximum absorbance. The absorbencies having maximum wavelength for CEFI and LEVO was selected.

Preparation of calibration curve

Aliquots of standard solutions of CEFI (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml) and LEVO (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 ml) were transferred in a series of 10 ml volumetric flask. The volume was adjusted to the mark with methanol and mixed.

The absorbance of all the solutions was measured at 230 nm and 298 nm against methanol as blank.

METHOD VALIDATION

Linearity

Linearity was observed in a concentration range of 2-24 µg/ml and 2 -14 µg/ml for CEFI and LEVO, respectively. The calibration curve was constructed by plotting the graph of absorbance Vs concentration.

Range

Range is the interval between upper and lower concentration of analyte for which it has been demonstrated that the analytical method has suitable level of precision, accuracy and linearity. The range for the method was observed in a concentration range of 2-24 µg/ml and 2-14 µg/ml for CEFI and LEVO, respectively. For the evaluation of the range accurately measured standard working solution of CEFI (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml) and LEVO (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 ml) was pipette out in to a separate series of 10 ml volumetric flask. The volume was adjusted with methanol and absorbance of all the solution was measured at 230 nm and 298 nm against methanol as blank.

Method precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measuring the absorbance of solutions (n = 6) of CEFI and LEVO (12 µg/ml for both drugs) without Changing the parameters of the simultaneous equation method. The results are reported in terms of relative standard deviation (% RSD).

Intermediate precision (Reproducibility)

The intraday and inter day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of CEFI and LEVO (2, 4, and 8 µg /ml). The results were reported in terms of relative standard deviation (% RSD).

Limit of detection (LOD) & Limit of Quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were calculated by using the following equations.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response S = slope of the calibration curve

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of CEFI and LEVO by the standard addition method. Known amounts of standard solution of CEFI and LEVO were added at 80 %, 100 % and 120 % levels to prequantified sample solutions of CEFI and LEVO.

Analysis of drugs in tablet formulation

The absorbance of sample solution was measured against methanol as blank at 230 and 298 nm for quantitation of CEFI and LEVO, respectively. The amount of CEFI and LEVO present in the sample solutions were determined by solving the simultaneous equations.

RESULTS AND DISCUSSION

Method development

The working standard solution of CEFI and LEVO were prepared separately in methanol. They were scanned in the wavelength range of 200-400 nm. Maximum absorbance was obtained at 230 nm and 298 nm for CEFI and LEVO, respectively. These two wavelengths were employed for the determination of CEF and LEV. Overlain spectra of both the drugs are shown in Figure 1.

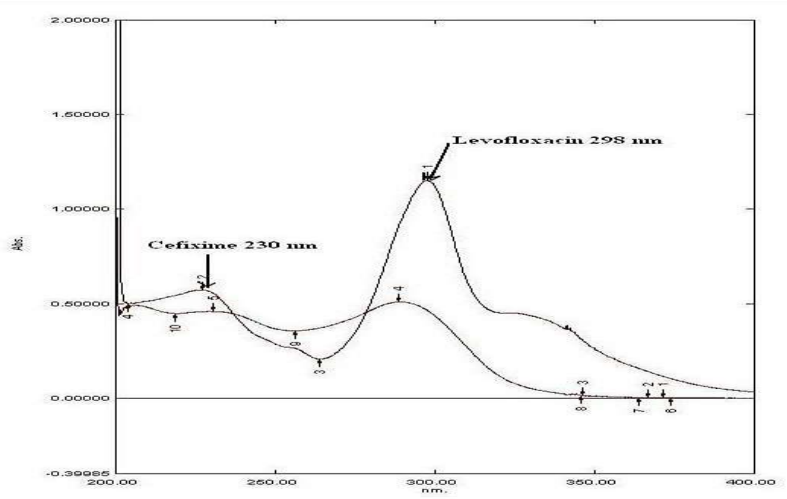


Fig: 1 Overlain UV absorption spectra of CEF (12 µg /ml) and LEV (12 µg /ml) in methanol

Validation of the simultaneous equations method

Linearity

Calibration range was observed in the concentration range of 2-24 µg /ml and 2-14 µg /ml respectively for CEFI and LEVO. The calibration curves at different wavelengths are shown in Figure.2,.3,.4,5

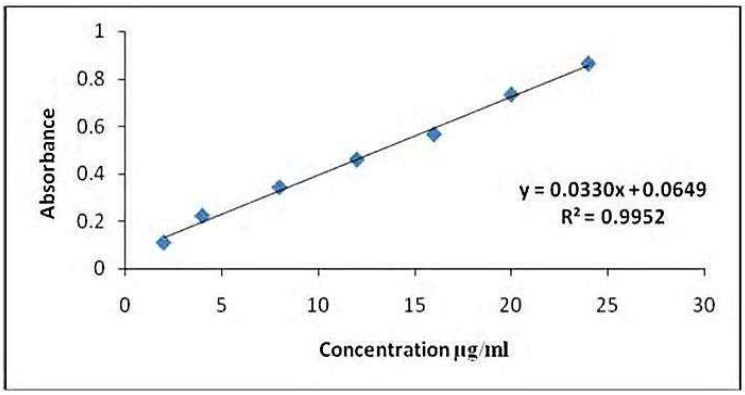


Fig 2: Calibration curve of CEFI at 230 nm

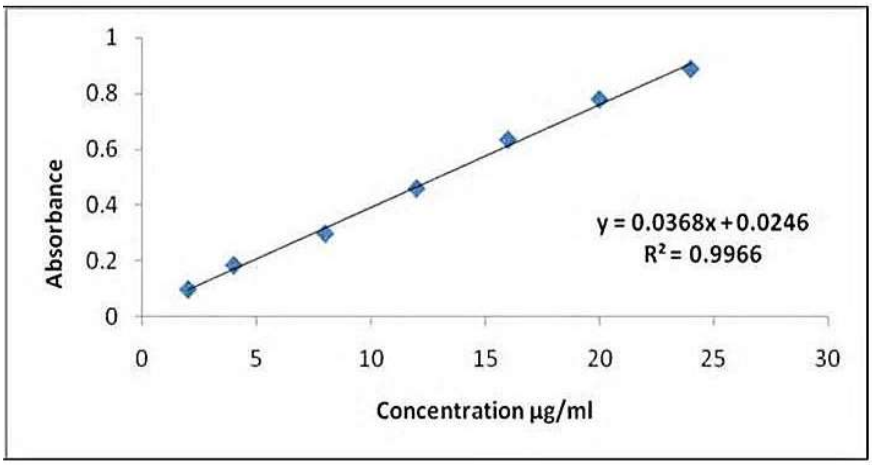


Fig 3: Calibration curve of CEFI at 298 nm

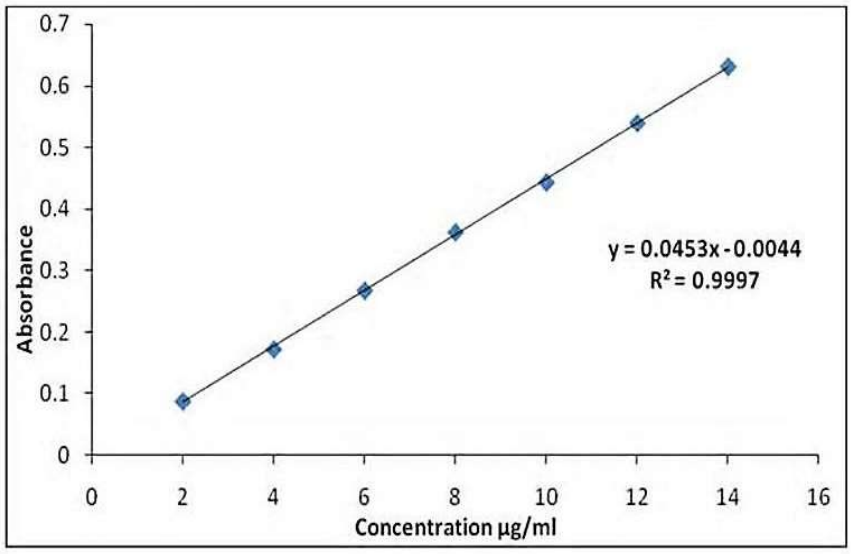


Fig 4: Calibration curve of LEVO at 298 nm

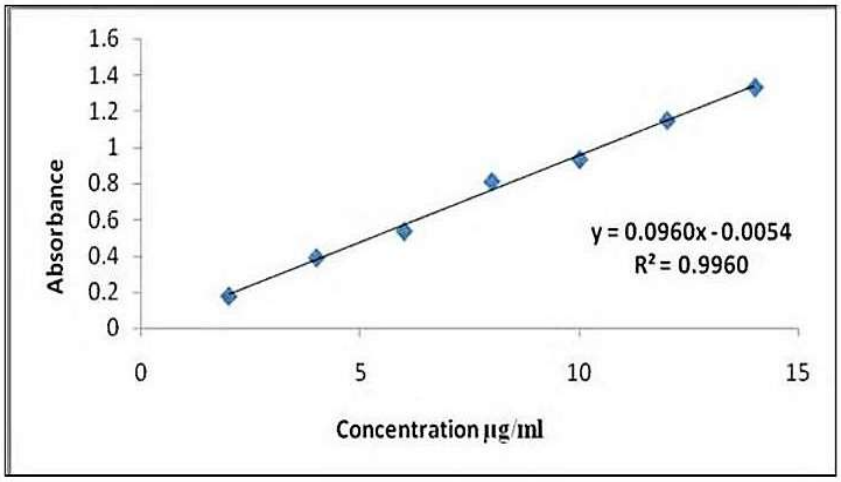


Fig 5: Calibration curve of LEVO at 230 nm

Table 1: Regression Analysis Data and Summary of Validation Parameter for the proposed Method

PARAMETERS		CEFI	CEFI	LEVO	LEVO
Wavelength (nm)		230	298	298	230
Beers law limit (µg /ml)		2-24	2-14	2-14	2-24
Regression equation Y=mX + c		Y = 0.033 x + 0.064	Y = 0.033 x + 0.0024	Y = 0.033 x + 0.0054	Y = 0.033 x + 0.0044
Slope		0.033	0.036	0.096	0.046
Intercept		0.064	0.0024	0.0054	0.0084
Correlation coefficient (r^2)		0.9952	0.9966	0.9960	0.9997
Repeatability (%RSD, n =6)		0.49	0.99	1.00	0.98
Precision (%RSD)	Intraday (%RSD)	0.37 – 0.55	0.13 -0.61	0.30 – 0.53	0.23 – 0.53
	Interday (%RSD)	0.49 -1.06	0.67 -0.59	0.40 – 0.84	0.14 – 0.29
LOD (µg /ml)		0.28	0.86	0.10	0.20
LOQ (µg /ml)		0.86	0.51	0.25	0.23
(Accuracy ± S.D) % Recovery n= 5		100.6 ± 0.7		100.7 ± 1.1	

Method precision (Repeatability)

The RSD values of CEF were found to be 0.49 and 0.99 % at 230.0 and 298.0 nm respectively. The RSD value of LEV was found to be 1.00 and 0.98 % at 298 and 230 nm (Table 2). Low value of RSD indicates that proposed method is repeatable.

Table 2: Precision data for CEF and LEV

Concentration (CEF : LEVO) (12 : 12 µg /ml)	CEFIXIME		LEVOFLOXACIN	
Wavelength (nm)	230 nm	298 nm	298 nm	230 nm
1	0.4584	0.4590	1.1504	0.5596
2	0.4592	0.2591	1.1554	0.5598
3	0.4631	0.2594	1.1531	0.5604
4	0.4598	0.4598	1.1542	0.5614
5	0.4604	0.4601	1.1563	0.5629
6	0.4624	0.4524	1.1582	0.5607
Mean	0.4605	0.4599	1.1546	0.5608
S.D	0.0018	0.0012	0.0027	0.0012
Repeatability (%RSD , n =6)	0.49	0.99	1.00	0.98

Intermediate precision (Reproducibility)

The RSD values of CEFI for interday (0.49-1.06 % and 0.67-0.592 %) and intraday (0.37-0.55 % and 0.13-0.61 %) at 230 and 298 nm, respectively and the RSD values of LEVO for interday (0.40-0.84and 0.14-0.29 %) and intraday (0.30-0.53 % and 0.23-0.73 %) at 298 nm and 230 nm for CEFI and LEVO reveals that the method is precise.

LOD and LOQ

LOD and LOQ values for CEFI were found to be 0.28 and 0.86 µg/ml, 0 .86 and 0.51 µg/ml at 230 and 298 nm, respectively. Where, LOD and LOQ values for LEVO were found to be 0.10 and 0.20 µg/ml, 0. 31 and 0.63 µg/ml at 2 98 nm and 230 nm, respectively. Low value of LOD & LOQ indicates that the method is sensitive. (Table 1)

Accuracy

The recovery experiments were performed by the standard addition method. The mean recoveries were found to be 100.6 ± 0. 7 and 100.7 ±1.1 for CEFI and LEVO respectively. The recoveries results indicate that the

proposed method is accurate. Results of recovery studies are shown in Table 3. S. D. is Standard deviation and n is number of replicate.

Table 3: Recovery data for the proposed method (n=3)

Drug	Amount present in formulation(µg /ml)	Amount added	% Recovery S.D (n =3)
CEFI	8.0	80	100.3 ± 0.8
	8.0	90	101.2 ± 0.8
	8.0	100	100.4 ± 0.1
LEVO	10.0	80	100.5 ± 0.8
	10.0	90	101.4 ± 0.2
	10.0	100	100.3 ± 1.5

S. D. is Standard deviation and n is number of replicate

Assay

The proposed validated method was successfully applied to determine CEFI and LEVO in tablet formulation. Results are given in Table 5.4. No interference of the recipients with the absorbance of analyte of interest appeared; hence the proposed method is suitable for the routine analysis of CEFI and LEVO in combined dosage forms.

Table 4: Analysis of CEF and LEV in tablet formulation by the proposed method (n = 6)

Tablet Formulation	Label claim (mg)		Amount find (mg)		% Label claim (mg) (n=6)	
	CEFI	LEVO	CEFI	LEVO	CEFI	LEVO
1	400	500	405	504	101.16	100.92
2	400	500	406.5	505.5	101.94	101.82
3	400	500	406.3	505.3	101.86	101.69
4	400	500	406	505.1	101.38	101.21
5	400	500	406	503.5	100.33	100.97
6	400	500	406.4	505.2	101.29	101.51
Mean			406.0	500.7	101.3	101.6
S.D			0.55	0.81	0.40	0.32

CONCLUSION

Based on the results, obtained from the analysis using proposed method, it can be concluded that the method has linear response in the range of 2-24 µg/ml and 2-14 µg/ml for CEFI and LEVO, respectively.

The result of the analysis of tablet formulation by the proposed method is highly reproducible and reliable and is in good agreement with label claim of the drugs. The additive present in the tablet formulation did not interfere in the analysis. So the method can be used for the routine analysis of drugs in combined dosage form.

REFERENCES

1. The united state pharmacopoeia drug information, Maryland, Rockville, MD, the United States pharmacopoeia convention. Inc. 2009: 2. p. 1835.
2. Rang HP, Dale MM, Ritter JN, Moore P. Pharmacology.5thed.New York: Churchill Livingstone;2005. p. 439-41.
3. Martindale. The complete drug reference.35thed. Vol. 1, 38.p.1736.
4. NeilMJ.The merkindex, anencyclopedia of chemicals, drugs&BioLogicals.13thed. p. 542.
5. Indian pharmacopoeia, Government of India. Vol. 2.New Delhi: Controller Publication;2010. p. 1012.
6. Tripathi KD. Essentials of medicalpharmacology.9thed; Jaypee Brother's Medical PublishersLtd.New Delhi;2008, p. 647,651,663,665.7. British pharmacopoeia, The British PharmacopoeiaCommission.6thed;2010(1):410.
8. Japanesepharmacopoeia. Vol. 443.Tokyo: Society of Japanese Pharmacopoeia, 15th edition2006, p. p. 938-39.
9. British Pharmacopoeia. LondonHerMajesty's Stationery Office,The BritishPharmacopoeiaCommission.6thed. Vol. 2; 2010.p. 1965.
10. United statepharmacopoeia 32, national formulary 27 states pharmacopoeia convention, Inc.Rockville, MD;2005. p. 3610.

11. ICHharmonizedTripartileguideline. Validation of analytical procedures: text and Methodology, International Conference on Harmonization, Geneva, Switzerland. Vol. Q2; 2005.(p. R1).
12. Anjaneyulu Y, Chandreshekhar K. A Text book of Analytical Chemistry.Pharmabooksyndicate;2006.p.312.
13. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry.14thed.CBS Publishers and New Delhi: Distributors; 1997. p.275-337.
14. Connors K. A textbook of Pharmaceutical analysis.3rded.John Wiley & Sons;1999.p. 221-4.
15. PandaSk, Sharma AK, Sahu LK.Indian J PharmSci. 2002;64(6):540-4.
16. Jain SK, Jain D, Tiwari M, Catered SC.Indian J PharmSci.2007;64(3):267-70.
17. Salinas F, Nevado JJ, Mansilla AE. A new spectrophotometric method for quantitative multicomponent analysis resolution of mixtures of Salicylic and salicyluric acids.Talanta.1990;37(3):347-51. doi: 10.1016/0039-9140(90)80065-n, PMID 18964949.
18. BerzasNevado JJ, GuiberteauCabanillas C, Salinas F. Spectrophotometric resolution of ternary mixtures of salicylaldehyde, 3-hydroxybenzaldehyde and 4- hydroxybenzaldehyde by the derivative ratio spectrumzero crossing method.Talanta.1992;39(5):547-53. doi: 10.1016/0039-9140(92)80179-h, PMID 18965415.
19. Swarbrick J, JamesB.C.Encyclopedia of pharmaceutical technology. Vol.I.New York: Marcel Dekker Inc; 1998. p. 217-24.
20. Lindsay S. HPLC by open learning.London: John wiley& sons; 1991. p. 30-45.
21. Lough WJ, Wainer W. HPLC fundamental principles and practices.Blackie Academic & Professional; 1991.p. 52-67.
22. Jeffery GH, Bassett J. Vogel's textbook of Quantitative Chemical Analysis.5thed; 1991. p. 217-35.
23. Sharma BK. Instrumental methods of chemical analysis.20thed.Goel Publishing House;2001.p.54-83.
24. Indian pharmacopoeia, Government of India. Vol. 2.New Delhi: The Controller Publication;2010. p. 1012.
25. Aysegul G, Borchu D, Sibel A. Voltametric determination of cefixime in dosage form and biological fluid.Talanta.2005;67:703-12.
26. Aysegul G, Borchu D, Sibel A. Voltametric determination of cefixime in dosage form and biological fluid.Talanta.2005;67:709-14.
27. Honda S, Taga A, Kakehi K, Koda S, Okamoto Y. Determination of cefixime& its metabolites by high performance capillary electrophoresis.J Chromatogr.1992;590(2):364-68. doi: 10.1016/0021-9673(92)85400-n, PMID 1560104.
28. Virypaxappa S, Shivaprasad K, Latha S. A simple method for the spectrophotometric determination of cefixime in pharmaceuticals.IntJ ChemEng Research;2010(1):23-30.
29. Eric-Jovanovic S, Agbaba D, Zivanov-Stakic D, Vladimirov S. EricS.Agbaba D, zivanov D, Vladimirov S. HPTLC determination of cephalosporins in dosage forms.J Pharm BiomedAnal.1998;18(4-5):893-98. doi: 10.1016/s0731-7085(98)00274-x, PMID 9919994.
30. Arshad HM, Gauhar S. Development of HPLC-UV method for analysis of cefixime in raw materials and in capsule. Jordan J PharmSci.2009;2(1):53-65.
31. Dhoka MV. Validated HPTLC method for determination of cefiximetrihydrateanderdosteine in bulk and combined pharmaceutical dosage. EurAsianJ AnalChem.2011;1:6.
32. Sharma R, Pathodiya G. Simultaneous estimation and validation of cefiximetrihydrate and ornidazole in bulk and tablets. J PharmRes.2010;3(12):953, 2955.
33. Rathinavel G, Mukherjee B. A validated RP-HPLC method for simultaneous estimation of cefixime and cloxacillin in tablets.E-journal of chemistry.2008;5(3):648-51.
34. Zendelovska D. HPLC method for determination of cefixime and cefotaxime in human plasma. Bull ChemTechnol Maced.2003;22(1):39-45.
35. Khan U, Sharif S, Ashfaq M, Asghar N. Simultaneous determination of potassium.
36. Dhoka MV. Validated HPTLC method for determination of cefiximetrihydrate and erdosteine in bulk and combined pharmaceutical dosage. EurAsianJ AnalChem.2011;7:9.
37. Deshpande MM, Kasture S, Gosavi SA. Application of HPLC and HPTLC for the simultaneous determination of cefiximetrihydrate and ambroxol hydrochloride in pharmaceutical dosage form.EurAsianJAnalChem.2010;5(3):227-2.
38. devika GS, Sudhakar M. Simultaneous estimation of cefixime and moxifloxacin in bulk and pharmaceutical dosage form by RP-HPLC. Orient J Chem.2012;28(4):1743-50.
39. Ajit R, Presahnt V. Simultaneous estimation of cefixime and cloxacilinbtRP-HPLC.Int J Pharm Arch.2010;1(2):317-20.
40. Patelbs, shivaprasad H, Latha S. A simple method for the spectrophotometric determination of cefixime and cefuroxime in pharmaceuticals.IntJ ChemEng Research.2010;1:23-30.
41. Desai P, Patel H, Ganesh, Pandey P,Kudaravalli S. Validation of RP-HPLC for the estimation of cefixime and cefdinir in oral suspension. IntJ Pharm Technol.June2010;2(2):385-95.

42. FalkowskiAJ, LookZM, Noguchi H, SilberBM. Determination of cefixime and lizenoid in biological samples by RP-HPLC. J Chromatogr.1987;422:145-52. doi: 10.1016/0378-4347(87)80447-4, PMID 3437003.
43. Indian pharmacopoeia, government of India, the controller publication, new Delhi.2010;2:3232.
44. Sun H, Li L, Chen X.Flow-injection chemiluminescence determination of ofloxacin and levofloxacin in pharmaceutical preparations and biological fluids.AnalSci.2006;22(8):1145-9. doi: 10.2116/analsci.22.1145, PMID 16896259.
45. Juan A.Cana, Manuelcallejon and FranciscoJose. Application of terbium sensitized luminescence to the determination of levofloxacin. BarraganAnal.2000;125:1851-4.
46. Altiokka G, Atkosar Z, Can NO. The determination of levofloxacin by flow injection analysis using UV detection, potentiometry and conductometry in pharmaceutical preparations.JPharmBiomedAnal.October2002;30(3):881-5. doi: 10.1016/s0731-7085(02)00354-0, PMID 12367715.
47. Santoro M, Kassabn NM, Singh K, Kedor M. Quantitative determination of, levofloxacin in pharmaceutical preparations by high-performance liquid chromatography. JPharmBiomedAnal.2006;40(1):179-84.
48. JuanA.Ocana J, BarraganManuelcallejon and Fernandode la Rosa. Application of lanthanide sensitized chemiluminescence to the determination of levofloxacin, moxifloxacin and trovafloxacin, j. MicrochimActa.January2004;144(1-3):207-13.
49. CaufieldWV, StewartJT. Determination of zidovudine and levofloxacin in human plasma by reversed phase HPLC and solid phase extraction. JLiqChromatogrRelatTechnol.2002;25(12):1791-805. doi: 10.1081/JLC-120005874.
50. Du LM, Yang YQ, Wang QM. Spectrofluorometric determination of certain quinolones through charge transfer complex formation.AnalChimActa.2004;516(1-2):237-43. doi: 10.1016/j.aca.2004.04.006.
51. Zhang C. Development and optimization of a reversed phase high performance liquid chromatographic method for the determination of levofloxacin in growth media, human bal fluid, alveolar macrophages and plasma. JInfectDisPharmacother;6(4):49-57. doi: 10.1300/J100v06n04_05.
52. Al-Momani IF. Flow injection spectrophotometric determination of the antibacteriallevofloxacin in tablets and human urine.AnalLett.March2006;39(4):741-50. doi: 10.1080/00032710600611186.
53. MeyyanathanSN, RamasarmaGVS, Suresh B. Analysis of levofloxacin in pharmaceutical preparations by high performance thin layer chromatography. JSepScience.December2003;26(18):1698-700. doi: 10.1002/jssc.200301616.
54. Altiokka G, Atkosar Z, Cann NO. The determination of levofloxacin by flow injection analysis using uv detection, in pharmaceutical preparations. JPharmBiomedAnal.October2002;30(3):886-7.
55. Santoro M, Kassab M, Singh K, Kedor M. Quantitative determination of, levofloxacin in pharmaceutical preparations by high-performance liquid chromatography.JPharmBiomedAnal.2006;40(1):189-94.
56. Luo S, Lei J, Zhang R, Cai H, Li R. Determination of levofloxacin in plasma and cerebrospinal fluid with HPLC and its pharmacokinetics in patients undergoing neurosurgical operations.Yao XueXueBao.December1998;33(12):937-40. PMID 12016861.
57. Sivasubramanian L, Sankar V.SSivaramanV ,Senthilkumar K, Muthulcumaran A, Raja K. Visible spectrophotometric determination of levofloxacin in tablet dosage forms. IndJPharmSci.2004;66(6):799-802. M.PHARM (Pharmaceutical Analysis).
58. FangM,XiayonC,ValinZ,DafangZ.Levofloxacininhumanplasma:applicationtoa pharmacokinetic study. J Chromatogr.2005;819:287-92.
59. SiewertS. Validation of levofloxacinHPLC assay in plasma and dialysate for pharmacokinetic studies. J PharmBiomedAnal.June2006;41(4):1360-2. doi: 10.1016/j.jpba.2006.02.010, PMID 16563690.
60. Shao X, Xiaodong, Xie, Xiaofeng, liu, yanhong set al.Rapid biological fluids using flow injection chemiluminescence. CurrAnalChem.July2006;2(3):253-9.
61. Neckel U, Joukhadar C, Frossard M, Jäger W, Müller M, Mayer BX. Simultaneous determination of levofloxacin and ciprofloxacin in microdialysates and plasma by high performance liqid chromatography. AnalChimActa.July2002;463(2):199-206. doi: 10.1016/S0003-2670(02)00429-4.
62. Satish A, NatvarlalJ. Development and validation of HPTLC method for simultaneous estimation of levofloxacin and ornidazole in tablet dosage form.J Pharm Educ Res. 2011;2(2).
63. Shafrose S, Haritha P. Validated simultaneous estimation and development of levofloxacin and ornidazole by RP-HPLC method. IntJ Pharm ClinRes.2012;4(4):52-5.